MAURI ORA 12: 159-169

METHODS FOR DISSECTION AND METAZOAN PARASITE EXAMINATION OF SMALL CETACEANS

F. CIPRIANO[1], D. BLAIR[2] and J. McKENZIE[2]

[1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona, 85721 U.S.A.

[2]Department of Zoology, University of Canterbury, Christchurch, New Zealand.

ABSTRACT

An outline of techniques and a brief, step-by-step protocol for measurement, dissection and parasite examination of small cetaceans are presented. Techniques include: standardised external morphometric examination, methods for sampling of tissues and organs, collecting and preserving parasites and stomach contents.

INTRODUCTION

During an ongoing study of dusky dolphins (Cipriano, 1985) at Kaikoura, New Zealand, a protocol was drawn up for examination and dissection of beach-cast or incidentally netted specimens. This protocol includes methods of collecting and preserving parasites, an aspect which is often ignored or treated superficially when small cetaceans are examined. However, we feel that study of parasites can provide information about the biology and individual histories of their hosts, and merits attention. The protocol presented here is brief. To compensate, we have provided references to more comprehensive works relating both to cetacean biology and to parasite collection and study. A data sheet for recording

measurements is included as an appendix. We hope the following is a helpful guide to anyone unfamiliar with cetaceans who is called upon to examine one of these animals.

PRECAUTIONS

Anyone working on cetaceans in New Zealand requires a permit from the Ministry of Agriculture and Fisheries, Wellington.

Marine mammals can be infected with Erysipelas, a bacterial disease which can be transmitted to humans and causes "Sealers' Thumb". This is an unpleasant chronic condition which is difficult to treat. Rubber gloves should ALWAYS be worn while handling unfixed material from cetaceans. Discarded tissues should be disposed of in an appropriate manner.

Although bones, especially ribs, may get in the way during dissections, it is best not simply to break them. Bone splinters can easily puncture gloves and cut fingers. Damaged bones reduce the quality of museum skeleton preparations. Ribs can be disarticulated at their junctions with the vertebral transverse spines.

If at all possible, animals should be examined while fresh. If immediate examination is not possible, they can be chilled (0-4°C) for a day or two with little deterioration. Freezing and thawing of carcasses greatly reduces the scientific value of tissue, stomach content and parasite samples. Animals should only be frozen as a last resort, not as a routine prelude to dissection.

MATERIALS AND METHODS

This protocol calls for the use of a minimal quantity of equipment. Suggested requirements are: Trays and bowls for temporary holding of organs, lidded containers for storage of samples, forceps, knives, scissors and scalpels for dissection, running water for washing, sieves, petri dishes, slides, cover slips, vaseline, salines, preservatives, tapemeasure, labels, pencils and a dissecting microscope.

Saline for field use can be made up as a 0.75% solution of NaCl OR as a mixture of 1 part seawater to 3 parts freshwater. Suggested preservatives are 70% alcohol and 10% formalin. Neutral buffered formalin is better than normal 10% formalin and can be made as follows: Dissolve 3.31g $NaH_2PO_4.H_20$ and 33.77g

$Na_2HPO_4.7H_2O$ in 1 l distilled water. To 1 part of concentrated formalin (40% formaldehyde) add 9 parts of this buffer.

COLLECTION OF STOMACH CONTENTS AND PARASITES

Stomach contents should be examined as soon as possible and parasites collected for preservation. After this, stomach contents can be preserved in alcohol (NOT formalin as this will tend to destroy otoliths) whereas parasites are best preserved in formalin. SEDIMENTATION is a useful technique to clean up voluminous stomach contents etc. and makes parasites easier to see. Place material in a bucket or large beaker. Add saline almost to the top and agitate a little to break up the mass of contents. Stir so that the entire contents of the container swirl gently in one direction. This will cause food items and parasites to accumulate in the centre at the bottom. Leave for 5-10 minutes. Gently pour off supernatant fluid (pour through a sieve if it is suspected that some food items are still in the supernate). Transfer sediment to a smaller container and repeat the process. Eventually the sediment will be clean enough to permit parasites to be seen. When those visible to the unaided eye have been picked out, the residue should be examined using a low powered dissecting microscope and appropriate illumination. In fresh material parasites can often be detected by their movement.

Many parasites are small and best found by scraping, with a blunt edge, the lining of the gastrointestinal tract and sedimenting the scrapings as above.

Organs such as the liver can be examined by slicing numerous times and examining cut surfaces for evidence of parasites. Slices of tissue can be washed vigorously in buckets of saline to flush out worms which can then be found in the sediment. Live worms will often move out of sliced tissues left for a while in saline.

FIXATION OF SPECIMENS

TISSUE SAMPLES should be preserved in formalin. Where small samples are kept, these should be cubes of about 1cm. Large samples (e.g. large testes) should be sliced lengthwise to allow rapid penetration of fixative. A foetus may need formalin injected into the body cavity to speed fixation.

WORMS collected should be fixed with care if the specimens are to be of any value. DEAD PARASITES (generally those from frozen and thawed carcasses) can be preserved by direct immersion in formalin. However, in the case of medium to large (>4mm) trematodes (except *Braunina*, see Fig. 1), a better preparation may

be obtained by holding them flat under a coverslip during fixation to minimise bending and distortion. Specimens should be placed flat on a slide with the suckers facing upwards. Hold them in this position by pressing down GENTLY on a coverslip supported by dabs of vaseline at the corners. Place slide in a petri dish (or similar) containing formalin which can penetrate under the coverslip to fix the specimen. After about one hour the specimen can be removed to a vial of formalin.

FRESH PARASITES that are observed, or suspected to still be alive, should be treated to prevent distortion during fixation. An easy general method suitable for ALL groups of parasites, and especially for small (<4mm) trematodes and *Braunina*, is as follows: Place the parasites in a very small volume of saline so that they are kept moist. QUICKLY add hot (60-70°C) formalin.

More specific methods, which are better for certain groups of parasites, are listed below:

TREMATODES	LARGE (>4mm) (except *Braunina*, see Fig. 1): Hold flat on slide with coverslip supported by dabs of vaseline at corners. Irrigate with formalin as outlined earlier.
CESTODES	Place in fresh water, preferably in refrigerator, until movement stops (several hours may be needed) then transfer into formalin. Attached worms should be teased away from tissues carefully to avoid damage to the scolex.
NEMATODES	Heat in dish of saline until motionless and virtually straight, then transfer to formalin. Do not overheat.
ACANTHOCEPHALANS	Place in fresh water until proboscis remains extruded, then transfer to formalin. It may be necessary to prick the cuticle with a needle to aid penetration of fixative.

DISSECTION PROTOCOL
(Remember to LABEL all material preserved)

WEIGHT	WEIGH carcass to nearest 0.5kg.
PHOTOGRAPHS	PHOTOGRAPH lateral and ventral aspects. Include rule in photo to provide scale.
MEASUREMENTS	MEASURE external proportions (see appendix). Measure blubber thickness mid-dorsal, lateral and ventral at same transverse plane just forward of dorsal fin edge.

EXTERNAL EXAMINATION	Examine skin surface for ectoparasites, diatom patches or any anomalies.
BLUBBER SAMPLE	Place carcass with LEFT side up. Starting at rear of genital slit, CUT forward along mid-ventral line 20% of total body length, then 10% of body length towards dorsal side to outline a rough rectangle. Carefully DISSECT AWAY rectangle of blubber from underlying tissue. Set aside for later examination.
FLENSE LEFT SIDE	FLENSE rest of left side. REMOVE flipper and scapula, separate from blubber and set aside. (DISSECT away blubber from underlying mammary tissue carefully).
MAMMARY SAMPLE	Make longitudinal SLICE through centre of left mammary. NOTE presence or absence of milk or colostrum (a thick viscous fluid). MEASURE maximum tissue thickness. COLLECT 5 cm square of tissue and PRESERVE in formalin.

(NOTE: IF FEMALE APPEARS TO BE PREGNANT GO TO APPROPRIATE STEP BELOW.)

LEFT GONAD	LABEL, then REMOVE left gonad. Set aside.
UROGENITAL TRACT	TIE OFF colon with string before cutting free. REMOVE urogenital tract; kidneys, ureters, bladder and urethra as a unit. Set aside.
RIGHT GONAD	LABEL, then REMOVE right gonad. Set aside.
PREGNANT FEMALES	Newly pregnant females: REMOVE entire reproductive tract with embryo, PRESERVE in formalin. Late-term pregnancy: REMOVE foetus from uterus, WEIGH, MEASURE, PHOTOGRAPH as for other animals. INJECT body cavity and PRESERVE in formalin.
GONAD WEIGHTS	WEIGH each gonad separately. PRESERVE in formalin.
INNOMINATES	REMOVE innominate (pelvic) bones. Set aside.
VISCERA	EXPOSE visceral cavity by disarticulating ribs. TIE OFF oesophagus just behind larynx. REMOVE viscera by cutting oesophagus and trachea, then dorsal aorta, mesenteries and diaphragm. Set aside for tissue sampling and parasite survey.

HEAD	REMOVE head by separating from the vertebral column at the junction of the condyles and the atlas vertebra. Set aside.
FLENSE RIGHT SIDE	ROLL carcass over and FLENSE right side. REMOVE flipper and scapula and set aside.
RIGHT MAMMARY	COLLECT 5 cm square of mammary tissue. LABEL and PRESERVE in formalin.
FLESH OUT	DISARTICULATE ribs. REMOVE remaining flesh. DIVIDE vertebral column into equal thirds by separating between vertebrae.
PREPARE SKELETON	PLACE skeletal parts in plastic mesh bags for initial cleaning by isopod "sea lice" or beetle colony.

PARASITE SURVEY

SKIN	After measuring specimen, EXAMINE entire external surface for attached crustaceans, lumps etc. COLLECT and PRESERVE samples in alcohol.
BLUBBER/FASCIA	EXAMINE rectangular blubber sample collected earlier by making parallel longitudinal slices 1 cm apart through blubber and into fascial layer. Separate strips. EXAMINE for cysts of *Phyllobothrium* (see Fig. 2) and count any found. COLLECT and PRESERVE cysts in formalin.
DIGESTIVE TRACT	DISSECT away oesophagus, stomach and intestine from rest of visceral mass and place in separate tray.
PANCREAS AND BILE DUCT	Follow bile duct to LOCATE pancreatic tissue in first loop of small intestine. CUT along bile duct and through pancreatic tissue to EXPOSE pancreatic ducts. EXAMINE ducts for parasites.
STOMACH	TIE OFF intestine just below third stomach, then detach and place intestine in separate tray. Float stomach in tub of saline so that contents and free parasites will settle to the bottom and not stick to stomach membranes.

	SEE CAUTIONS ABOVE concerning preservation methods.
	OESOPHAGUS AND FORESTOMACH: CUT along oesophagus and first stomach and lay open. EXAMINE and COLLECT parasites and food items as outlined earlier.
	SECOND STOMACH: CUT open and EXAMINE as above COUNT *Braunina* (see Fig. 1) and remove by cutting free small piece of stomach lining to which each parasite is attached.
	THIRD STOMACH: DISSECT away second stomach, then CUT open third stomach. EXAMINE as before. FIX fresh stomach lining and attached *Braunina* in hot formalin, or if carcass has been frozen, in cold formalin. INCLUDE with lining any *Braunina* from second stomach.
INTESTINE	CUT open short lengths at a time. EXAMINE as outlined earlier. In the case of frozen and thawed carcasses, it is also possible to FLUSH through the entire intestine with water into a tub and recover parasites and food items from the sediment.
RESPIRATORY TRACT	CUT open trachea, bronchi and bronchioles and EXAMINE. SLICE sections of lung tissue and carefully DISSECT out any worms embedded in lung parenchyma. If necessary CUT out a block of tissue containing lungworm head capsule for later sectioning.
MIDDLE EAR AND NASAL SACS	CUT away the hyoids from their attachment to the base of the skull. Carefully EXAMINE the cavity surrounding the ear bones and pick out exposed parasites. Thoroughly flush saline or water through the nasal sacs into a tray. EXAMINE the tray contents for parasites.
LIVER	EXAMINE as outlined earlier.
KIDNEYS AND UROGENITALS	SLICE open urethra into bladder, lay open and EXAMINE. CUT open ureters up through collecting ducts and EXAMINE.
HEART	EXAMINE as outlined earlier for liver.

ACKNOWLEDGEMENTS

Many of the dissection procedures and the blubber survey method were developed from techniques taught by W.A. Walker to one of us (F. Cipriano) during training as a Dall's porpoise observer at the National Marine Mammal Laboratory in Seattle, Washington. The observer training programme developed by Linda Jones and Larry Tsunoda at NMML provided an appreciation of attention to detail and logical consistency that prompted the development of these procedures for our own use.

BIBLIOGRAPHY

CIPRIANO, F. 1985. Dusky dolphin research at Kaikoura, New Zealand: a progress report. *Mauri Ora 12*: 151-158.

DELYAMURE, S.L. 1955. *Helminthofauna of Marine Mammals (Ecology and Phylogeny)*. Translated from Russian by Israel Program for Scientific Translations, Jerusalem, 1968. 522 pp.

FRASER, F.C. 1952. *Handbook of R.H. Burne's Cetacean Dissections*. British Museum of Natural History, London. 70 pp.

PRITCHARD, M.H. and KRUSE, G.O.W. 1982. *The Collection and Preservation of Animal Parasites*. University of Nebraska Press, Lincoln and London. 141 pp.

RIDGEWAY, S.H. (ed.) 1972. *Mammals of the Sea*. Charles C. Thomas, Illinois. 812 pp.

WINCHELL, J.M. 1982. *Field Manual for Small Cetacean Dissection*. College of the Atlantic, Bar Harbor, Maine. 65 pp.

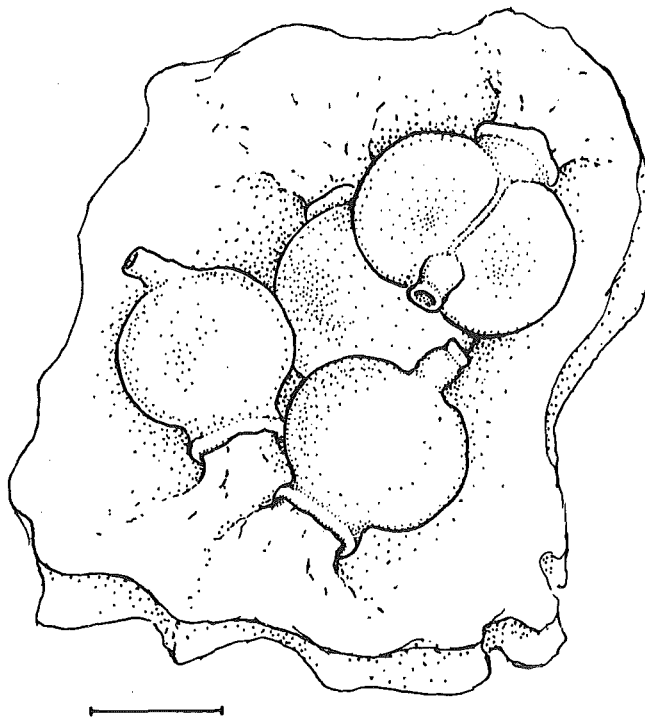


Figure 1. Group of adult *Braunina cordiformis* attached to stomach lining of dusky dolphin. (Scale bar = 5 mm).

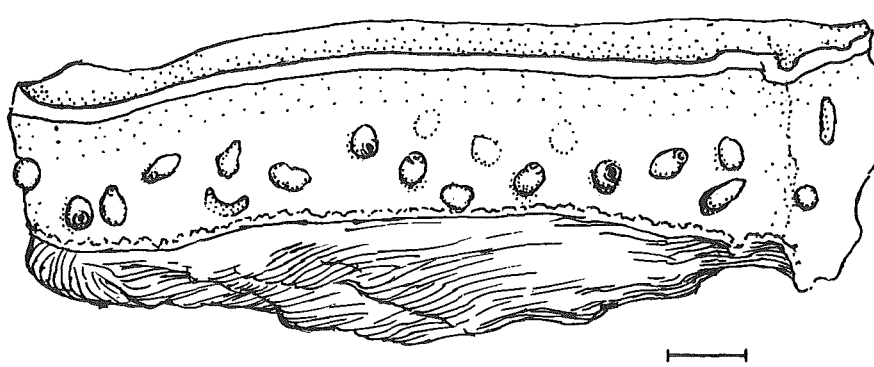


Figure 2. Slice through skin, blubber, and underlying muscles of dusky dolphin, showing appearance of *Phyllobothrium delphini* cysts. (Scale bar = 10 mm).

Drawings by Stephen Tyerman.

CETACEAN MEASUREMENT RECORD

SPECIES_____ SEX_____ WEIGHT (KG)_____ SPECIMEN NO._____

DATE/TIME COLLECTED_____ BY_____

COLLECTION CONDITION? FRESH/DECOMPOSED FROZEN AFTER COLLECTION? YES/NO

LOCALITY_____

DATE DISSECTED_____ BY_____

PHOTOGRAPHS? YES/NO BY_____

NOTE: ALL LONGITUDINAL MEASUREMENTS TAKEN IN A STRAIGHT LINE PARALLEL TO BODY AXIS

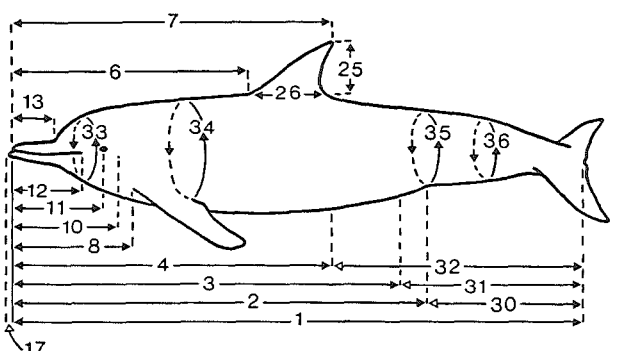
UNITS OF MEASUREMENT: CM UNLESS OTHERWISE INDICATED

KEY: U. = UPPER; L. = LOWER; (R) = RIGHT; (L) = LEFT

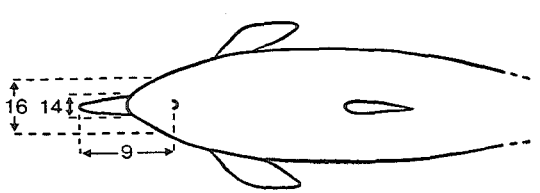
1. TOTAL LENGTH: U. JAW - FLUKE NOTCH_____
2. U. JAW - CENTRE ANUS_____
3. U. JAW - CENTRE GENITAL SLIT_____
4. U. JAW - CENTRE UMBILICUS_____
5. L. JAW - END VENTRAL GROOVE_____
6. U. JAW - LEAD EDGE DORSAL FIN_____
7. U. JAW - TIP DORSAL FIN_____
8. U. JAW - ANT. INSERTION FLIPPER (R)_____
9. U. JAW - CENTRE BLOWHOLE_____
10. U. JAW - AUDITORY MEATUS (R)_____
11. U. JAW - CENTRE EYE (R)_____
12. U. JAW - ANGLE GAPE_____
13. U. JAW - APEX MELON_____
14. MAX. WIDTH ROSTRUM_____
26. LENGTH DORSAL FIN BASE_____
27. FLUKE SPAN_____
28. FLUKE WIDTH_____
29. FLUKE NOTCH DEPTH_____
30. FLUKE NOTCH - CENTRE ANUS_____
31. FLUKE NOTCH - CENTRE GENITAL SLIT_____
32. FLUKE NOTCH - UMBILICUS_____
33. GIRTH AT EYE_____
34. GIRTH AT AXILLA_____
35. GIRTH AT ANUS_____
36. GIRTH MIDWAY ANUS - FLUKE NOTCH_____
37. BLUBBER THICKNESS: MIDDORSAL_____
38. BLUBBER THICKNESS: LATERAL_____
39. BLUBBER THICKNESS: MIDVENTRAL_____

15. LENGTH THROAT GROOVES_____
16. CENTRE EYE - CENTRE EYE_____
17. PROJECTION L. JAW BEYOND U. JAW_____
18. EYE HEIGHT (R)_____
19. EYE LENGTH (R)_____
20. BLOWHOLE WIDTH_____
21. BLOWHOLE LENGTH_____
22. FLIPPER WIDTH_____
23. FLIPPER LENGTH: TIP - ANT. INSERTION (R)_____
24. FLIPPER LENGTH: TIP - AXILLA (R)_____
25. DORSAL FIN HEIGHT_____

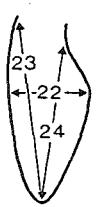
40. U. TOOTH COUNT (R)_____
41. U. TOOTH COUNT (L)_____
42. L. TOOTH COUNT (R)_____
43. L. TOOTH COUNT (L)_____
44. U. BALEEN COUNT (R)_____
45. U. BALEEN COUNT (L)_____
46. LENGTH LONGEST BALEEN PLATE_____
47. MAMMARY SLIT LENGTH (R)_____
48. MAMMARY SLIT LENGTH (L)_____
49. ANAL SLIT LENGTH_____
50. GENITAL SLIT LENGTH_____



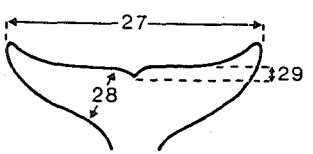
LATERAL VIEW



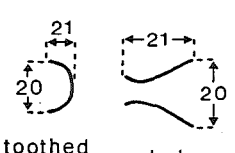
DORSAL VIEW



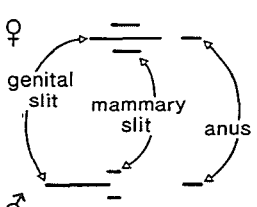
FLIPPER



FLUKES



toothed baleen
BLOWHOLES



UROGENITAL SLITS